for each test group.) (A) Total cell number—Two weeks after reconstitution cell numbers were decreased and there was no significant difference in cell number between castrated and noncastrated mice. Four weeks after reconstitution cell numbers were approaching normal levels in castrated mice. (B) $CD45.2^+$ cell number—There was no significant difference between castrated and noncastrated mice with respect to $CD45.2^+$ cell number in the spleen, two weeks after reconstitution. $CD45.2^+$ cell number remained high in castrated mice at four weeks. There were no donor-derived cells in the noncastrated mice at the same time point.

[0045] Figure 23 A-C: Splenic T cells and myeloid and lymphoid derived dendritic cells (DC) after fetal liver reconstitution. (n=3-4 mice for each test group.) Control (white) bars on the following graphs are based on the normal number of T cells and dendritic cells found in untreated age matched mice. (A) T cell number—Numbers were reduced two and four weeks after reconstitution in both castrated and noncastrated mice. (B) Donor derived ($CD45.2^+$) myeloid dendritic cells—two and four weeks after reconstitution DC numbers were normal in both castrated and noncastrated mice. At two weeks there was no significant difference between numbers in castrated and noncastrated mice. (C) Donor-derived ($CD45.2^+$) lymphoid dendritic cells—numbers were at normal levels two and four weeks after reconstitution. At two weeks there was no significant difference between numbers in castrated and noncastrated mice.

REMARKS

Claims:

Original claims 1 – 14 are cancelled by this amendment. Claims 15 – 54 are added.

Amendments to Specification:

The amendments to the specification are made to correct typographical errors and for clarity. For example, the word "or" was inadvertently left out of paragraph 0068. In paragraph 0068, support for "Eulexin" can be found in the original claim 13. The

description for Figures 1 through 23 have been amended to correct typographical errors such as discrepancies between what the figure legends recited and what is shown on the figures. For example, missing p values and statistical error indicators. The amended Description of Figures clarifies what is presented in the figures without changing any of the information provided by the figures. Applicant requests that the amendments be accepted as they merely correct typographical errors and clarify the meaning of the figures.

Restriction Requirement:

Prior to this amendment, claims 1-14 were pending. Claims 1-10 and 14 as Group I and claims 1-9 and 11-14 as Group II, are subject to a restriction requirement based upon Examiner's view that Group I claims are drawn to a method for improving vaccination wherein the method of disrupting sex steroid mediated signaling to the thymus is through surgical castration and Group II claims being drawn to a method for improving vaccination wherein the method of disrupting sex steroid mediated signaling to the thymus is through administration of pharmaceutical

Original claims 1 – 14 are cancelled by this amendment. Claims 15 - 54 have been added by this amendment. Applicant hereby elects without traverse Group II. Applicant believes that all of claims 15 – 54 fall within Group II. However, Applicant hereby reserves the right to prosecute claim 10 and similar or related claims in continuation and/or divisional applications.

Accordingly, claims 15 - 54 are under consideration in the above-identified patent application. No new matter has been added. Support for claims 15 - 54 is found throughout the specification.

Species Election:

The Examiner further requests under 35 U.S.C. 121(1) that Applicant elect a single disclosed species to which the claims would be restricted if no generic claim is finally held to be allowable, and to list all claims readable thereon including those subsequently added. The Examiner has not indicated nor identified the species from

which Applicant must choose, which would be considered consonant with the requirement. Applicant is unclear as to the species election and respectfully requests the Examiner to clarify the species election requirement.

Conclusion

The Applicant respectfully asserts that all pending claims are in condition for allowance and requests that the Examiner allow claims 15 – 54.

If the Examiner feels for any reason that direct contact with Applicants' attorney will advance the prosecution of this case, the Examiner is invited to contact the undersigned attorney at the number given below.

The Commissioner is hereby authorized to charge payment of additional claims and additional filing fees or credit any overpayment to Deposit Account No. 09-0946.

Respectfully submitted,

IRELL & MANELLA LLP

Dated: 5.14.03

By: _____
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Reg. No. 46, 618

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IN THE DESCRIPTION OF THE FIGURES:

[0023] Figure 1 A and B: Changes in thymocyte number pre- and post-castration. Thymus atrophy results in a significant decrease in thymocyte numbers with age. Aged (2-year old) mice were surgically castrated and analysed for (A) thymus weight in relation to body weight and (B) total cells per thymus, at 2-4 weeks post-castration. A significant decrease in thymus weight and cellularity was seen with age compared to young adult (2-month) mice. This was restored by castration. [By 2 weeks post-castration, cell numbers have increased to young adult levels. [By 3 weeks] At 3-weeks post-castration, [numbers have significantly increased from the young adult and they are stabilized] thymic hypertrophy was observed and was returned to young adult levels by 4 weeks post-castration. Results are expressed as mean + 1SD of 4-8 mice per group. ** = $p< 0.01$; ***= [Significantly different from young adult (2 month) thymus,] $p<0.001$ compared to young adult and post-castration mice.

[0001] Figure 2 A-C: Aged (2-year old) mice were surgically castrated and analysed at 2 and 4 weeks post-castration for peripheral lymphocyte populations. (A) Total lymphocyte numbers in the spleen. Spleen numbers remain constant with age and post-castration. (B) The ratio of B cells to T cells did not change with age or post-castration. [The B:T cell ratio in the periphery also remains constant (B),] however[, the CD4:CD8 ratio decreases significantly ($p<0.001$) with age and is restored to normal young levels] (C) a significant decrease in the CD4+:CD8+ T cell ratio was seen with age. This was restored by 4 weeks post-castration. Data is expressed as mean +1SD of 4-8 mice per group. *** = $p<0.001$ compared to young adult (2-month) and 4-week post-castrate mice.

[0002] Figure 3: Fluorescence Activated Cell Sorter (FACS) profiles of CD4 vs. CD8 thymocyte populations with age and post-castration. [Percentages for each quadrant are given above each plot. Subpopulations of thymocytes remain constant with age and there is a synchronous expansion of thymocytes following castration.] Aged (2-year old) mice were castrated and the thymocyte subsets analysed based on

the markers CD4 and CD8. Representative FACS profiles of CD4/CD8 dot plots are shown for $CD4^-CD8^-$DN, $CD4^+CD8^+$DP, $CD4^+CD8^-$ and $CD4^-$/CD8 and $CD4^-CD8^+$ SP thymocytes. No difference was seen in the proportions of any CD4/CD8 defined subset with age or post-castration.

[0003] Figure 4: [Proliferation of thymocytes as detected by incorporation of a pulse of BrdU. Proportion of proliferating thymocytes remains constant with age and following castration.] Aged (2-year old) mice were castrated and injected with a pulse of bromodeoxyuridine (BrdU) to determine levels of proliferation. Representative histogram profiles of the proportion of $BrdU^+$ cells within the thymus with age and post-castration are shown. No difference in the proportion of proliferating cells within the total thymus was observed with age or post-castration.

[0004] Figure 5 A-D: Effects of age and castration on proliferation of thymocyte subsets. (A) Proportion of each subset that constitutes the total proliferating population—The proportion of CD8+ T cells within the proliferating population is significantly increased. (B) [Percentage of each subpopulation that is proliferating—The TN and CD8 Subsets have significantly less proliferation at 2 years than at 2 months. At 2 weeks post-castration, the TN population has returned to normal young levels of proliferation while the CD8 population shows a significant increase in proliferation. The level is equivalent to the normal young by 4 weeks post-castration. (C) Overall TN proliferation remains constant with age and post-castration.] However, a significant decrease in the proportion of DN ($CD4^-CD8^-$) thymocytes proliferating was seen with age. Post-castration, this was restored and a significant increase in proliferation within the $CD4^-CD8^+$ SP thymocytes was observed. (C) No change in the total proportion of $BrdU^+$ cells within the TN subset was seen with age or post-castration. However (D) the significant decrease in proliferation of the TN1 ($CD44^+CD25^-$) subpopulation with age is not returned to normal levels by 4 weeks post-castration. Results are expressed as mean +1SD of 4-8 mice per group. * = $p<0.05$; ***=[Highly significant, $p<0.001$,] $p<0.001$ compared to young adult (2-month) mice. [**=significant, $p<0.01$,]

[0005] Figure 6 A-C: [Mice] Aged (2-year old) mice were castrated and were injected intrathymically with FITC to determine thymic export rates. The number of

FITC+ cells in the periphery were calculated 24 hours later. [Although the proportion of recent thymic migrants (RTE) remained consistently about 1% of thymus cell number age but was significantly reduced at 2 weeks post-castration, there was a significant ($p<0.01$) decrease in the RTE cell numbers] (A) A significant decrease in recent thymic emigrant (RTE) cell numbers was observed with age. Following castration, these values [were increasing although still significantly lower than young mice at 2 weeks post-castration. With age, a significant increase in the ratio of CD4+ to CD8+ RTE was seen and this was normalized by 1 week post-castration.] had significantly increased by 2 weeks post-cx. (B) The rate of emigration (export/total thymus cellularity) remained constant with age but was significantly reduced at 2 weeks post-cx. (C) With age, a significant increase in the ratio of CD4+ to CD8+ RTE was seen and this was normalised by 1-week post-cs. Results are expressed as mean +1SD of 4-8 mice per group. ** = $p<0.01$; *** = $p < 0.001$ compared to young adult mice. ^ = $p <0.001$ compared to castrated mice.

[0006] Figure 7 A-C: Changes in thymus (A), spleen (B) and lymph node (C) cell numbers following treatment with cyclophosphamide, a chemotherapy agent. [Note the rapid expansion of the thymus in castrated animals when compared to the non-castrate (cyclophosphamide alone) group at 1 and 2 weeks post-treatment. In addition, spleen and lymph node numbers of the castrate group were well increased compared to the cyclophosphamide alone group. By 4 weeks, cell numbers are normalized. (n = 3-4 per treatment group and time point).] Young (3-month old) mice were depleted of lymphocytes using cyclophosphamide. Mice were either sham-castrated or castrated on the same day as cyclophosphamide treatment. (A) A significant increase in thymus cell number was observed in castrated mice compared to sham-castrated mice. (B) Castrated mice also showed a significant increase in spleen cell number at 1-week post-cyclophosphamide treatment. (C) A significant increase in lymph node cellularity was also observed with castrated mice at 1-week post-treatment. Results are expressed as mean +1SD of 4-8 mice per group. *** = $p<0.001$ compared to castrated mice.

[0007] Figure 8 A-C: Changes in thymus (A), spleen (B) and lymph node (C) cell numbers following irradiation [(625 Rads) one week after surgical castration.] and castration on the same day. Note the rapid expansion of the thymus in castrated animals when compared to the non-castrate [(irradiation alone)] group at [1 and] 2 weeks post-treatment. [(n = 3-4 per treatment group and time point).] No difference in spleen (B) or lymph node (C) cell numbers was seen with castrated mice. Lymph node cell numbers were still chronically low at 2-weeks post-treatment compared to control mice. Results are expressed as mean 1SD of 4-8 mice per group. * = $p<0.05$ compared to control mice; *** = $p<0.001$ compared to control and castrated mice.

[0008] Figure 9 A-C: Changes in thymus (A), spleen (B) and lymph node (C) cell numbers following irradiation [and castration on the same day. Note the rapid expansion of the thymus in castrated animals when compared to the non-castrate group at 2 weeks post-treatment. However, the difference observed is not as obvious as when mice were castrated 1 week prior to treatment (Fig. 7). (n = 3-4 per treatment group and time point).] (625 Rads) one week after surgical castration (A). No difference in spleen (B) or lymph node (C) cell numbers was seen with castrated mice. Lymph node cell numbers were still chronically low at 2-weeks post-treatment compared to control mice. Results are expressed as mean + 1SD of 4-8 mice per group. + = $p < 0.05$; ** = $p < 0.01$ compared to control mice; *** = $p < 0.001$ compared to control and castrated mice.

[0009] Figure 11 A and B: Lymph node cellularity following foot-pad immunization with Herpes Simplex Virus-1 (HSV-1). Note the increased cellularity in the aged post-castration as compared to the aged non-castrated group (A). Bottom graph illustrates the overall activated cell number as gated on CD25 vs. CD8 cells by FACS (B).

[0010] Figure 12 A-C: Vβ10 expression on CTL (cytotoxic T lymphocytes) in activated LN (lymph nodes) following HSV-1 inoculation. Despite the normal V□10 responsiveness in aged mice overall, in some mice a complete loss of V□10 expression was observed. Representative histogram profiles are shown. Note the diminution of a

clonal response in aged mice and the reinstatement of the expected response post-castration.

[0011] Figure 13 A-C: Castration restores responsiveness to HSV-1 immunization. [(a)] (A) Aged mice showed a significant reduction in total lymph node cellularity post-infection when compared to both the young and post-castrate mice. [(b)] (B) Representative FACS profiles of activated ($CD8^+CD25^+$) cells in the LN of HSV-1 infected mice. No difference was seen in proportions of activated CTL with age or post-castration. [(c)] (C) The decreased cellularity within the lymph nodes of aged mice was reflected by a significant decrease in activated CTL numbers. Castration of the aged mice restored the immune response to HSV-1 with CTL numbers equivalent to young mice. Results are expressed as mean ±1 SD of 8-12 mice. ** = $p \leq 0.01$ compared to young (2-month) and non-castrated [mice; ^ = $p \leq 0.01$ compared to aged (non-cx)] mice.

[0012] Figure 14: Popliteal lymph nodes were removed from mice immunized with HSV-1 and cultured for 3 days. CTL assays were performed with non-immunized mice as control for background levels of lysis (as determined by ^{51}Cr-release). Results are expressed as mean of 8 mice, in triplicate ±1SD. Aged mice showed a significant ($p \leq 0.01$, *) reduction in CTL activity at an E:T ratio of both 10:1 and 3:1 indicating a reduction in the percentage of specific CTL present within the lymph nodes. Castration of aged mice restored the CTL response to young adult levels. * = $p < 0.01$ compared to young adult and post-castrate aged mice.

[0013] Figure 15 A and B: Analysis of $CD4^+$ T cell help and Vβ TCR response to HSV-1 infection. Popliteal lymph nodes were removed on D5 post-HSV-1 infection and analysed ex-vivo for the expression of (a) CD25, CD8 and specific TCRVβ markers and (b) CD4/CD8 T cells. [(a)] (A) The percentage of activated ($CD25^+$) $CD8^+$ T cells expressing either Vβ10 or Vβ8.1 is shown as mean ±1SD for 8 mice per group. No difference was observed with age or post-castration. [(b)] (B) A decrease in CD4/CD8 ratio in the resting LN population was seen with age. This was restored post-castration. Results are expressed as mean±1SD of 8 mice per group. *** = $p \leq 0.001$ compared to young and castrate mice.

[0014] Figure 19 A and B: Myeloid and lymphoid dendritic cell (DC) number after lethal irradiation, fetal liver reconstitution and castration. (n= 3-4 mice for each test group.) Control [(striped)] (white) bars on the following graphs are based on the normal number of dendritic cells found in untreated age matched mice. (A) Donor-derived myeloid dendritic cells—Two weeks after reconstitution DC were present at normal levels in noncastrated mice. There were significantly more DC in castrated mice at the same time point. (*$p \leq 0.05$). At four weeks DC number remained above control levels in castrated mice. (B) Donor-derived lymphoid dendritic cells—Two weeks after reconstitution DC numbers in castrated mice were double those of noncastrated mice. Four weeks after treatment DC numbers remained above control levels.

[0015] Figure 21 A-C: Changes in T cells and myeloid and lymphoid derived dendritic cells (DC) in bone marrow of castrated and noncastrated mice after fetal liver reconstitution. (n=3-4 mice for each test group.) Control [(striped)] (white) bars on the following graphs are based on the normal number of T cells and dendritic cells found in untreated age matched mice. (A) T cell number—Numbers were reduced two and four weeks after reconstitution in both castrated and noncastrated mice. (B) Donor derived myeloid dendritic cells—Two weeks after reconstitution DC cell numbers were normal in both castrated and noncastrated mice. At this time point there was no significant difference between numbers in castrated and noncastrated mice. (C) Donor-derived lymphoid dendritic cells—Numbers were at normal levels two and four weeks after reconstitution. At two weeks there was no significant difference between numbers in castrated and noncastrated mice.

[0016] Figure 22 A and B: Change in total and donor ($CD45.2^+$) [spleen] lymph node cell numbers in castrated and noncastrated mice after fetal liver reconstitution. (n=3-4 mice for each test group.) (A) Total cell number—Two weeks after reconstitution cell numbers were decreased and there was no significant difference in cell number between castrated and noncastrated mice. Four weeks after reconstitution cell numbers were approaching normal levels in castrated mice. (B) $CD45.2^+$ cell number—

There was no significant difference between castrated and noncastrated mice with respect to $CD45.2^+$ cell number in the spleen, two weeks after reconstitution. $CD45.2^+$ cell number remained high in castrated mice at four weeks. There were no donor-derived cells in the noncastrated mice at the same time point.

[0017] Figure 23 A-C: Splenic T cells and myeloid and lymphoid derived dendritic cells (DC) after fetal liver reconstitution. (n=3-4 mice for each test group.) Control [(striped)] (white) bars on the following graphs are based on the normal number of T cells and dendritic cells found in untreated age matched mice. (A) T cell number—Numbers were reduced two and four weeks after reconstitution in both castrated and noncastrated mice. (B) Donor derived ($CD45.2^+$) myeloid dendritic cells—two and four weeks after reconstitution DC numbers were normal in both castrated and noncastrated mice. At two weeks there was no significant difference between numbers in castrated and noncastrated mice. (C) Donor-derived ($CD45.2^+$) lymphoid dendritic cells—numbers were at normal levels two and four weeks after reconstitution. At two weeks there was no significant difference between numbers in castrated and noncastrated mice.